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The essential oil of *Croton zehntneri* and *trans*-anethole improves cutaneous wound healing

Josenilda Malveira Cavalcanti [a], José Henrique Leal-Cardoso [a], Lúcio Ricardo Leite Diniz [b], Viviane Gomes Portella [a], Carolina Oliveira Costa [a], Cybelle Façanha Barreto Medeiros Linard [a], Karuza Alves [c], Maria Valdenir Abreu de Paula Rocha [a], Crystiane Calado Lima [a], Vânia Marilande Cecatto [a], Andrelina Noronha Coelho-de-Souza [a,*]

[a] *Department of Physiology, Superior Institute of Biomedical Sciences, State University of Ceará, Av. Paranjana, 1700, 60740-000 Fortaleza, CE, Brazil*
[b] *Chisthus Faculty of Medicine, Fortaleza, CE, Brazil*
[c] *Department of Odontology, Federal University of Ceará, Fortaleza, CE, Brazil*

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ABSTRACT

Ethnopharmacology relevance: *Croton zehntneri* is a *Euphorbiaceae* species native to northeastern Brazil, where teas and beverages made from *Croton zehntneri* leaves are used as healing agents. To our knowledge, there is no experimental study supporting this claim of pharmacological activity.
Materials and methods: Full-thickness excisional wounds were made in the left and right sides of the dorsum of anesthetised Swiss mice, and a topical pharmaceutical formulation, developed by including essential oil extracted from the leaves of *Croton zehntneri* (2% and 20% EOCz) in pluronic-127 (PF-127), was administered to mice twice daily for 15 days post-wounding. To evaluate the contribution of *trans*-anethole, the major constituent of EOCz (85.7%), in the wound healing activity of EOCz, the effect of the topical administration of *trans*-AT on wound tissue repair was also evaluated and compared to other groups. A macroscopic analysis of swelling and exudates was performed and scored as 0 (missing), 1 (light), 2 (moderate) and 3 (intense). The number of capillaries and leukocytes was counted in hematoxylin and eosin (HE)-stained sections of the injured tissue. For extracellular matrix remodelling analysis, fibroblasts and collagen fibres present in the photomicrography of the Masson's Trichrome (MT)-stained sections were counted. Each experimental group comprised six mice.
Results: At day 3 post-wounding, it was observed that treatment with 20% EOCz greatly reduced the swelling and exudates with a similar magnitude to the dexamethasone treatment. The inflammatory cell infiltration and angiogenesis were not altered by either the EOCz- or *trans*-AT treatments. In contrast, an acceleration of the wound closure was observed, with an enhanced number of fibroblasts and collagen fibres in both the 20% EOCz- and *trans*-AT-treated mice.
Conclusion: Our data indicate that EOCz exerts significant wound healing activity, demonstrating its relevant therapeutic potential.

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1. Introduction

Croton zehntneri (*Euphorbiaceae*) is a bush native to the drought ecosystem of the *caatinga* from the northeast Brazil, where it is commonly known as *canela de cunhã*, *canela de cheiro* and *canelinha* (Craveiro et al., 1980; Leal-Cardoso and Fonteles, 1999). Preliminary findings have demonstrated that the *Croton zehntneri* leaves have a rich essential oil content that usually comprises 2–4% of the weight of dry leaves, varying with the hour of the day of the leaf collection, the season of the year and the region within northeast Brazil (Craveiro et al., 1994). *Trans*-anethole (AT) is the major constituent found in the essential oil of *Croton zehntneri* (EOCz) and is closely implicated with the pharmacologic activity attributed to EOCz (Albuquerque et al., 1995; Batatinha et al., 1995; Leal-Cardoso and Fonteles, 1999; Chainy et al., 2000; Oliveira et al., 2001; Carvalho et al., 2003; Rodrigues et al., 2009; Coelho-de-Souza et al., 2012). In indigenous medicine, teas and beverages made with the leaves of *Croton zehntneri* are used in the treatment of gastrointestinal disorders, as well as for anxiety and anorexia (Lazarini et al., 2000; Morais et al., 2006; Rodrigues et al., 2009; Coelho-de-Souza et al., 2012). In addition, it has been claimed by local herbal dealers that the decoctions and cataplasms of the *Croton zehntneri* leaves are also used as local anti-inflammatory and healing agents (Matos et al., 1991; Leal-Cardoso and Fonteles, 1999).

* Corresponding author. Tel.: +55 85 31019828; fax: +55 31 31019796.
E-mail address: andrelinanoronha@gmail.com (A.N. Coelho-de-Souza).

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To scientifically justify these claimed therapeutic effects and provide safe and efficient drugs for the treatment of different diseases, our group has investigated the pharmacological effects of EOCz and its constituents, including AT, over the past few years. For example, we have shown that EOCz and AT significantly reduced the muscular contractile activity of the intestinal smooth muscle and enhanced the production of the gastric wall mucus layer, suggesting antispasmodic and gastroprotective effects (Coelho-de-Souza et al., 2012). In skeletal muscle, we observed that EOCz and AT blocked the excitation–contraction coupling, released Ca^{++} from the sarcoplasmic reticulum and induced contraction (Albuquerque et al., 1995). In the cardiovascular system, our group found that EOCz treatment induced an initial hypotension followed by a pressor response in normal conscious rats, whereas it induced a bradycardia accompanied by a depressor reflex in anaesthetised rats, which appears to be mediated by the activation of vallinoid TPRV1 receptors located on the sensory vagal nerves (Lahlou et al., 1999; de Siqueira et al., 2006).

The analgesic and anti-inflammatory effects are important pharmacologic properties attributed to EOCz and AT (Chainy et al., 2000; Oliveira et al., 2001; Fontenelle et al., 2008). In addition, Rodrigues et al. (2009) verified that EOCz increased the effect of the antibiotic gentamicin. It is known that these above-mentioned activities act synergistically in the healing process itself (Velnar et al., 2009). Open cutaneous wounds remain one of the major complications of various diseases, such as diabetes and venous ulcers (Abu-Al-Basal, 2010; Atiba et al., 2011). Because open wounds increase the vulnerability to invasion of injured tissue by microorganisms, creating a vicious cycle with subsequent devastating consequences (Kumar et al., 2007; de Fatima et al., 2008), safe and efficient wound healing agents are needed to accelerate wound closure. Thus, based on these facts, we hypothesised that EOCz and AT may be wound healing agents, and we undertook this investigation to elucidate this hypothesis. Here, we demonstrated and characterised the healing effects of EOCz and AT and partially elucidated their mechanisms of action.

2. Materials and methods

2.1. Commercial drugs

Dexamethasone (Dexason®, Laboratório Teuto Brasileiro S/A, Goiás, Brazil), bovine fibrinolysin (fibrase®, Laboratório Pfizer Ltd., São Paulo, Brazil), *trans*-anethole (Sigma-Aldrich, St. Louis, MO, USA), pluronic F-127 (Sigma Chemical Co., St. Louis, MO, USA), xylazine (Rompum®, Bayer Schering Pharma, São Paulo, Brazil), ketamine (Dopalen®, Vetbrands, São Paulo, Brazil) and polyvinylpyrrolidone-iodine (Laboratório Madrevita Ltd., Ceará, Brazil) were acquired from the indicated suppliers.

2.2. Animals

Swiss mice (25–30 g) were obtained from the bioscience unit of the Christus Faculty, Fortaleza, CE, Brazil. They were housed in standard conditions with free access to standard chow and water. The animals were kept at room temperature (22 ± 2 °C) with a light/dark cycle of 12/12 h. All procedures described had prior approval from the local animal ethics committee (no. 07227619-3).

2.3. Plant material

The leaves of *Croton zehntneri* were collected in September 1998 near the city of Viçosa, CE, Brazil. The identification of the plants was confirmed by Dr. FJ Abreu Matos (Laboratory of Natural Products, Federal University of Ceará). A voucher specimen (no. 277477) was deposited in the herbarium of Prisco Viana, Federal University of Ceará.

2.3.1. Extraction and chromatographic analysis of the essential oil of *Croton zehntneri* (OECz)

The essential oil of *Croton zehntneri* (OECz) was isolated from freshly chopped leaves by steam distillation and analysed chemically as previously described (Oliveira et al., 2001). Chemical characterisations of OECz were performed by GC/MS. The chromatographic analysis was carried out on a Hewlett-Packard 6971 using the following analytical conditions: a dimethylpolysiloxane DB-1 fused silica capillary column (30 m × 0.25 mm; 0.1 μm); helium (1 mL/min) as a carrier gas; 250 °C injector temperature; 200 °C detector temperature; a column temperature of 35–180 °C at 4 °C/min and then 180–250 °C at 10 °C/min; and mass spectra of electronic impact 70 eV. These compounds were identified using a mass spectral library search and ^{13}C-NMR spectroscopy.

The composition of the essential oil from the leaves of *Croton zehntneri* (EOCz) used in this study has been previously described by Oliveira et al. (2001); briefly, the composition of OECz was 85.7% anethole, 4.8% estragole, 2.95% 1,8-cineole, 2.2% *trans*-caryophyllene, and 2.23% unidentified compounds.

2.3.2. Preparation of topical pharmaceutical formulation

Pluronic F-127 (PF-127) gels (10%w/w), a known thermoreversible gel used in pharmaceutical formulations for topical delivery systems, were prepared by slowly adding a pre-weighed amount of PF-127 to distilled water, followed by vigorous manual agitation for 3–5 min. After homogenisation, these gels were kept in closed dark bottles under refrigeration. The presence of pluronic in the formula allowed the formation of a thin surface layer over wounds, avoiding the wasting of doses and increasing the oil dispersion. Appropriate amounts of EOCz or *trans*-AT were added to the cold solution of PF-127, resulting in topical pharmaceutical formulations of EOCz or AT on concentrations of 2% (2% EOCz and 2% AT, respectively) and 20% (20% EOCz and 20% AT, respectively).

2.4. Experimental wounding

Swiss mice were anesthetised with ketamine and xylasine at doses of 60 mg/kg and 10 mg/kg, respectively, by intramuscular injection. The dorsal region was trichotomised, sterilised with a solution of polyvinylpyrrolidone-iodine, and full-thickness excisional wounds were made in two sites (left and right sides) of the dorsum of each animal using a sterile biopsy punch.

2.5. Drug administration

The wounds at the dorsum of each mouse were divided into two sections, one serving as a control (left side) in which the wounds were treated only with Pluronic 127 and the other (right side) treated with gel formulations containing EOCz or *trans*-AT at concentrations of 2% or 20%, twice daily, for 3 to 15 days post-wounding.

A saline solution (0.9% NaCl) incorporated in pluronic 127 was used as a control, and commercial topical formulations containing dexamethasone (1 mg) and bovine fibrinolysin (5 mg) were used as negative and positive controls, respectively. Each experimental group comprised six mice.

2.6. Measurement of the wound closure

The wounds of each mouse were photographed digitally (Mitsuca digital camera DS7373BR) at 0, 3 and 15 days post-wounding. The digital photographs of the cutaneous wounds were transferred

to a computer and converted into the Tagged Information File Format (TIFF) using the Scion Image 4.0.3.2 software. These images were processed and used to measure the wound area. The analyses for the wound areas were performed using the Scion Image for Windows, Release Beta 4.0.2 analysis program. The average area of wounds was expressed as a percentage of the initial area (day 0) using the equation:

2.7. Measurement of swelling and exudates

The cutaneous wounds were macroscopically analysed, and the magnitudes of the swelling and exudates were classified in a blind manner according the following scores: 0 (missing), 1 (light), 2 (moderate) and 3 (intense).

2.8. Histomorphological analysis

For histomorphological analysis, the formalin-fixed tissues were dehydrated through a graded alcohol series, cleared in a xylene series and embedded in paraffin. Serial sections (5 μm thick) from the paraffin-embedded regenerated tissues were cut by microtome and stained with hematoxylin-eosin (HE) and Masson's trichrome (MT). The number of blood vessels and inflammatory cells were counted in the HE-stained sections. The severity of the inflammation in the healed areas was evaluated in a blind manner by counting the inflammatory cell infiltration per field for six samples in each group.

The presence of fibroblasts and collagen fibers were evaluated by analysis of the photomicrography of MT-stained sections, in which the nuclei and collagen fibers are stained black and blue, respectively, whereas muscle, erythrocytes and the cell cytoplasm are stained red. The MT-stained sections were examined under the optical microscope and representative sections were photographed using Olympus ® model CH 30, adapted with ocular histometric Karl Zeiss Jena® model GF-P 10 ×. The quantification of the collagen content was performed by counting all of the collagen fibers, regardless of thickness. The regeneration of healed areas of evaluated by counting the mean number of fibroblast and collagen fibers per five randomised fields (× 400 magnification) of six samples in each group.

2.9. Statistical analysis

All the results are expressed as the mean ± the standard error of the mean (S.E.M.). The significance ($P < 0.05$) of the results was assessed by one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison tests.

3. Results

3.1. Effects of the essential oil of Croton zehntneri (EOCz) and trans-AT during the inflammatory phase (day 3 post-wounding) of the wound healing process

In terms of the percentage of tissue regeneration (Table 1), EOCz treatment did not produce any significant improvement in the wound healing (42 ± 1.68 and 54 ± 3.03%, 2 and 20% EOCz, respectively, $n=6$ for each group, Fig. 1E), compared to control and fibrinolysin treatments (51 ± 1.67 and 53 ± 0.3%, respectively, $n=6$ for each group, Fig. 1A and C), after three days of treatment. During same period, a reduced wound closure rate was observed in the *trans*-AT-treated mice (39 ± 1.82 and 25 ± 2.43%, 2 and 20% *trans*-AT, respectively, $n=6$ for each group, Fig. 1F), which was not statically different to that produced by the dexamethasone treatment (19 ± 3.35%, $n=6$, Fig.1D).

In the macroscopic analysis of injured tissues, it was observed that the 20% EOCz-treated mice showed an approximate 88%

Table 1
Comparison of the average wound size and residual wound area between days 0 and 3 post-wounding.

Day		Control	Fibrinolysin	Dexa	2% EOCZ	20% EOCZ	2% AT	20% AT
0	Wound size (mm^2)	4.12 ± 0.38	3.68 ± 0.31	3.01 ± 0.20	4.16 ± 0.22	4.10 ± 0.16	3.45 ± 0.16	3.6 ± 0.18
	Wound closure (%)	0	0	0	0	0	0	0
3	Wound size (mm^2)	2.02 ± 0.21	1.84 ± 0.17	2.49 ± 0.11^{+}	2.43 ± 0.17	1.87 ± 0.16	2.23 ± 0.14*	2.8 ± 0.17^{+}
	Wound closure (%)	51 ± 1.67	53 ± 0.90	19 ± 3.34^{+}	42 ± 1.68	54 ± 3.03	39 ± 1.82*	25 ± 2.43^{+}

Difference between the control and tested groups.
$^{*}P < 0.05$; $^{+}P < 0.0001$.

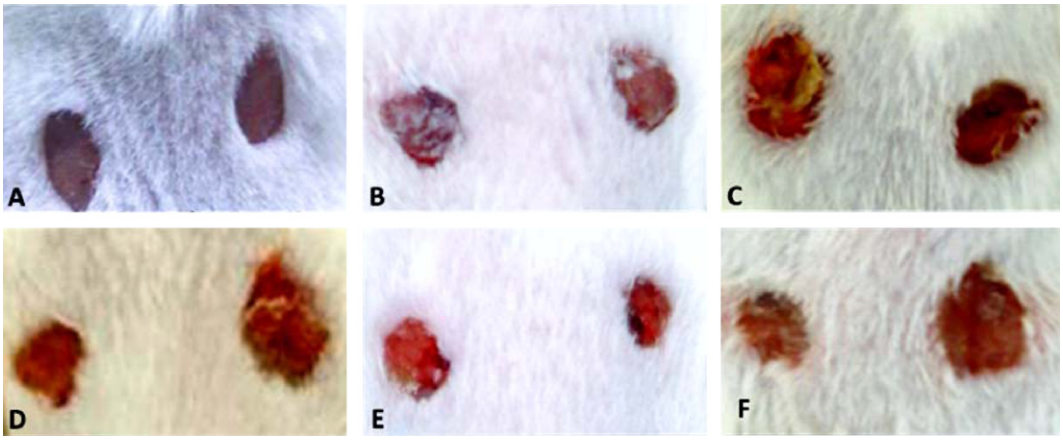

Fig. 1. Effects of the essential oil of *Croton zehntneri* (EOCz) and anethole (AT) on wound closure on day 3 post-wounding. (A) Control, day 0; (B) control, day 3; (C) fibrinolysin; (D) dexamethasone; (E) 20% EOCz and (F) 20% AT groups.

reduction in swelling (0.33 ± 0.21 compared to 2.6 ± 0.21 in the control group, $n=6$ for each group) and exudates (0.17 ± 0.17 compared to 2.3 ± 0.21 in the control group, $n=6$ each group), which was equal to the dexamethasone-treated mice (0.33 ± 0.21 and 0.17 ± 0.17, swelling and exudates scores, respectively, $n=6$ for each group) and higher than the fibrinolysin-treated mice (1.5 ± 0.22 and 2.0 ± 0.2, swelling and exudates scores, respectively, $n=6$ for each group), as seen in Fig. 2A. Except for the *trans*-AT (AT) treatments, which increased the infiltration of the inflammatory cells (23.7 ± 1 and 24.83 ± 0. 48 cells/fields, 2 and 20% AT, respectively, compared to 19.7 ± 1 in the control, $n=6$ for each group) (Fig. 2C), no significant changes in the number of blood vessels (Fig. 2B) or inflammatory cells (Fig. 2C) in the HE-stained sections from the injured tissue of the experimental groups were observed. As expected in this initial phase of the wound healing process, no differences in the fibroblast cells or collagen fibres were found in the MT-stained sections from the injured tissue among the experimental group animals (Fig. 2D).

3.2. Effects of the essential oil of Croton zehntneri (EOCz) and trans-anethole during the remodelling phase (day 15 post-wounding) of the wound healing process

As shown in the Table 2, a large reduction in the wound area of the 20% EOCz-treated mice (93 ± 0.39 vs. 72 ± 1.30% in the control group, $n=6$ for each group), which was similar to that produced by the fibrinolysin treatment (93 ± 0.25%, $n=6$), indicating a wound healing potential of the herbal product, after 15 days of treatment. In addition, the 20% *trans*-AT treatment induced significantly enhanced wound closure (90 ± 1.73%, $n=6$). As illustrated in Fig. 3, the appearance of the healed areas in the mice subjected to the 20% EOCz (Fig. 3D) and 20% *trans*-anethole (Fig. 3E) treatments was reduced in comparison to control group (Fig. 3A) and almost identical to that of the fibrinolysin-treated mice (Fig. 3B) at day 15 post-wounding. No significant differences in the number of blood vessels or inflammatory cells were observed among experimental groups

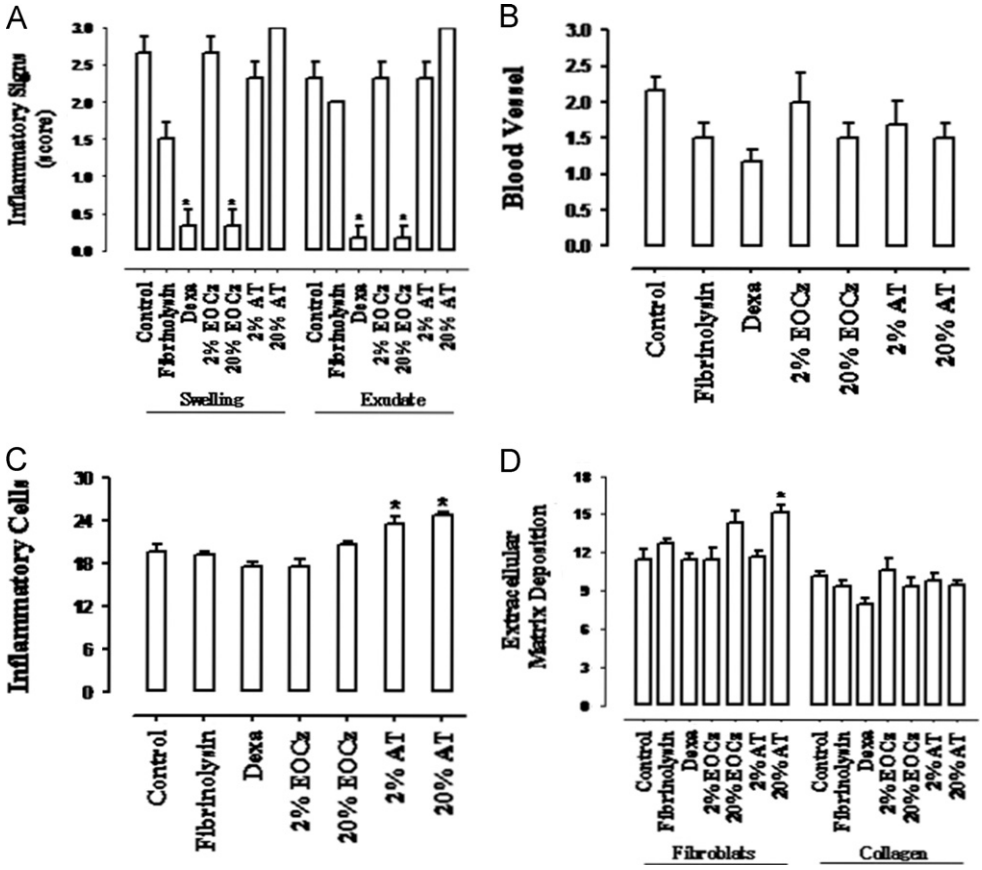

Fig. 2. Evaluation of the wound healing process on day 3 post-wounding from the following groups ($n=6$, mice/group): control group, fibrinolysin, dexamethasone, 2 or 20% OECz- and anethole-treated mice. (A) Swelling and exudates, (B) blood vessels, (C) inflammatory cells and (D) collagen fibres and fibroblasts. Data represent the mean ± SEM.

Table 2
Comparison of the average wound size and residual wound area between days 0 and 15 post-wounding.

Day		Control	Fibrinolysin	Dexa	2% EOCZ	20% EOCZ	2% AT	20% AT
0	Wound size (mm^2)	4.12 ± 0.38	3.68 ± 0.31	3.01 ± 0.20	3.63 ± 0.21	3.39 ± 0.18	3.25 ± 0.27	3.02 ± 0.13
	Wound closure (%)	0	0	0	0	0	0	0
15	Wound size (mm^2)	1.15 ± 0.14	$0.23 \pm 0.02^+$	$2.48 \pm 0.12^+$	1.03 ± 0.07	$0.21 \pm 0.01^+$	0.79 ± 0.06	$0.31 \pm 0.06^+$
	Wound closure (%)	$72\% \pm 1.30$	$93\% \pm 0.25^+$	$18\% \pm 3.82^+$	$70\% \pm 2.97$	$93\% \pm 0.39^+$	$75\% \pm 2.12$	$90\% \pm 1.73^+$

Difference between the control and tested groups. $^+P < 0.0001$.

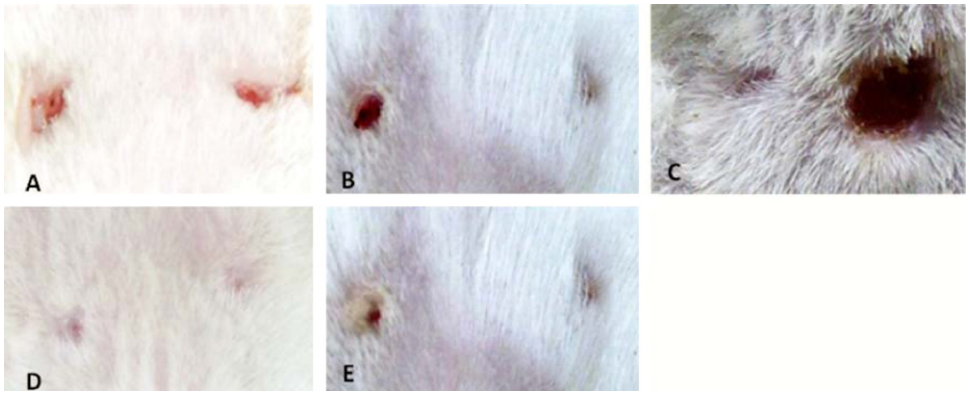

Fig. 3. Effects of the essential oil of *Croton zehntneri* (EOCz) and anethole (AT) on wound closure on day 15 post-wounding. (A) Control, day 0; (B) control, day 15; (C) fibrinolysin; (D) dexamethasone; (E) 20% EOCz and (F) 20% AT groups.

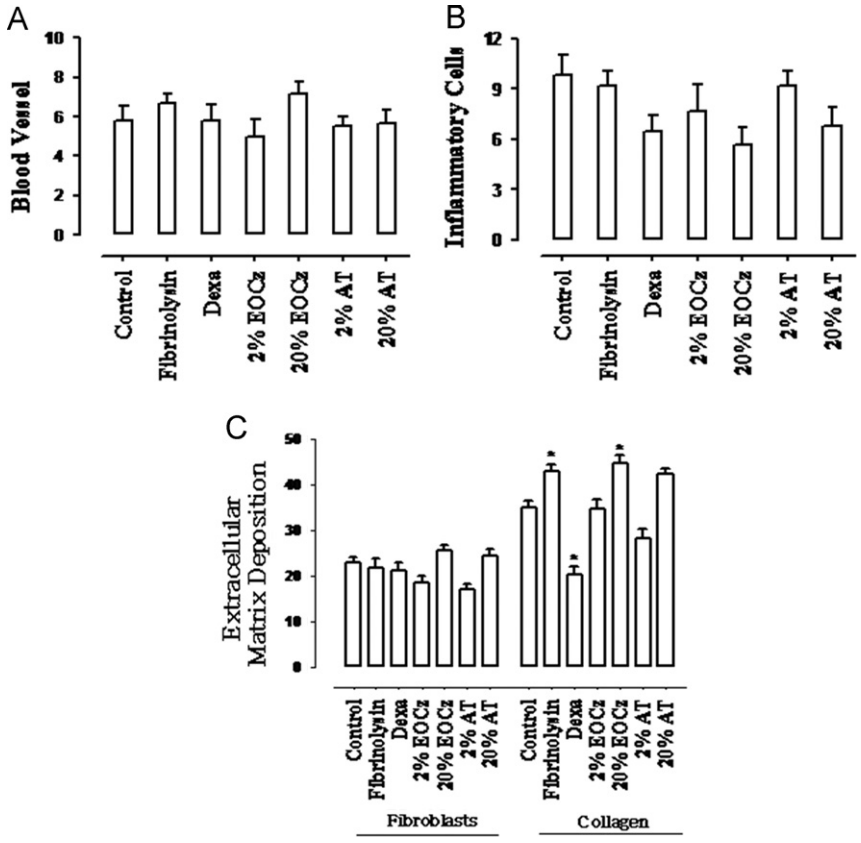

Fig. 4. Evaluation of the wound healing process on day 15 post-wounding from the following groups ($n=6$, mice/group): control, fibrinolysin, dexamethasone, 2 or 20% OECz- and anethole-treated mice. (A) Blood vessels, (B) inflammatory cells (C) collagen fibres and fibroblasts cells. Data represent the mean ± SEM.

(Fig. 4A and B). However, treatments with 20% EOCz, as well as with 20% *trans*-anethole, promoted a significant increase in the number of collagen fibres (44.83 ± 1.51 and 42.33 ± 1.42, respectively, $n=6$ for each group) compared to the control and dexamethasone-treated animals (35.0 ± 1.3 and 20.17 ± 1.78, respectively, $n=6$ for each group) (Fig. 4C).

3.3. Histomorphological analysis

The histomorphological examination of hematoxylin-eosin (HE)—stained sections prepared from the wounds of control, 20% EOCz and 20% AT-treated mice 3 (Fig. 5A and C) and 15 (Fig. 5D and I) days post-wounding exhibited the following characteristics. The tissue section of control animals showed pressence of crust, newly formed epithelium, granulation tissue, dermis intact, keratinised stratified squamous epithelium with presence of inflammatory infiltrate 3 days post-wounding (Fig. 5A) whereas continuous keratinised epithelium, presence of spindle-shaped fibroblasts and absence of inflammatory cells with a great amount of collagen fibers arranged homogeneously in the superficial and deep regions of lesioned tissue were found in the HE and MT-stained sections (Fig. 5D and G, respectively) of untreated mice 15 days post-wounding. The analysis of the sections of 20% OECz-treated mice showed thick-epithelium with moderated inflammatory infiltrated, intense vascularisation, presence of loose connective tissue with reduced spindle-shaped fibroblasts three days post-wounding (Fig. 5B). However, evidences of the progressive regeneration of the lesioned tissue, characterised by presence of keratinised epithelium, well-organised connective tissue accompanied by reduced granulated tissue and inflammatory cells, might be found in the sections of

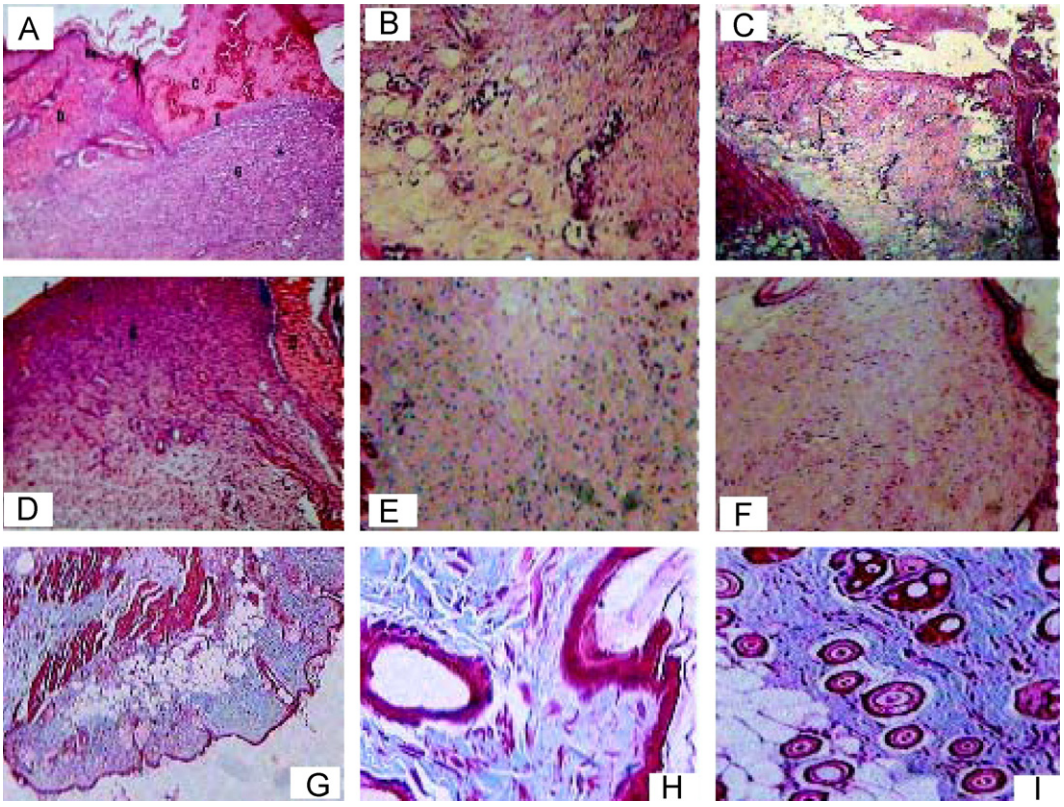

Fig. 5. Effects of the essential oil of *Croton zehntneri* (EOCz) and anethole (AT) on histomorphological structure. Control, 3 (A) and 15 (D) days post-wounding; HE staining; 40 ×, (C) crust, (E) newly formed epithelium, (G) granulation tissue, (D) dermis intact, (Eq) keratinised stratified squamous epithelium, absence of inflammatory infiltrate. 20% OECz, 3 (B) and 15 (E) days post-wounding HE staining; 100X. 20% AT, 3 (C) and 15 (F) days post-wounding HE staining; 400 ×. (G) Control, (H) 20% EOCz, (I) 20% AT, 15 days post-wounding, MT staining; 40 ×. Collagen fibers and fibroblasts stained in blue and red, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

20% EOCz-treated animals (Fig. 5E and H) 15 days post-wounding. The Fig. 5C shows that 20% AT-treated animals exhibited intense inflammatory infiltrate, extracellular oedema and loose connective tissue arranged heterogeneously 3 days post-wounding whereas a well-organised connective tissue with scattered inflammatory cells and granulation tissue (Fig. 5F) accompanied by great amount of spindle-shaped fibroblasts and collagen fibbers bundles (Fig. 5I) are present in histological sections of 20% AT group 15 days post-wounding.

4. Discussion

The most important finding of this current study is that topical administration twice daily for 15 days of the essential oil extracted from the leaves of *Croton zehntneri* (EOCz) accelerated the cutaneous wound closure rate in the experimental excision wound model in mice. In addition, we showed that anethole (AT), the major constituent of EOCz (85.7% w/w), promoted a wound healing activity very similar to EOCz, suggesting that AT is closely linked to the EOCz healing effect.

In the last several years, the wound healing effects of the essential oils extracted from medicinal plants have been reported (Kumar et al., 2007, de Fatima et al., 2008). Tumen et al. (2011) showed that the essential oils from *Cedrus libani* and *Abies cicilica* induced remarkable wound healing and anti-inflammatory activities on the linear incision and circular excision experimental wound models in rats. Similarly, Suntar et al. (2011) have attributed the potent wound healing activity of the *Hypericum perforatum* and oregano essential oils to the combined interactions of the essential oil components.

The restoration of the architecture and the functional integrity of the injured tissue involves complex and overlapping processes, including inflammation, wound contraction, angiogenesis, extracellular matrix deposition and tissue remodelling. All these process are integrated and occur in a timely manner during the inflammatory, proliferative and remodelling phases of wound healing. Single or multiple mechanisms may play a role in any one or more of the individual phases of wound healing, contributing to the overall outcome of the wound repair process (Velnar et al., 2009; Grieb et al., 2011).

At day 3 post-wounding, the initial inflammatory phase of the wound healing process, a decrease in the swelling and exudates in mice treated with 20% EOCz was observed. The effects on the swelling and exudates were similar to dexamethasone treatment and higher than the fibrinolysin treatment (Fig. 2A). Although no changes in the number of leukocytes was induced by EOCz at day 3 post-wounding (Fig. 2C), a reduction in the number of leukocytes was observed in the HE-stained sections from the injured tissue of the mice treated with 20% EOCz at day 15 post-wounding, similar to the dexamethasone-treated mice (Fig. 4B). In spite of the absence of effect on the swelling and exudates observed in the AT-treated animals (Fig. 2A), anti-inflammatory activity of AT by inhibition of the TNF-induced cellular response has been reported by Chainy et al. (2000).

In addition, other bioactive compounds are present in the composition of EOCz, such as estragole and 1.8-cineole, and they might contribute, in isolation or by interacting with AT, in the reduction of swelling and exudates observed in the EOCz-treated group (Fig. 2A). Furthermore, in a previous study, our group showed that oral administration of EOCz to mice increased the latency time in a hot-plate test and decreased the paw licking

time in the formalin test, suggesting an anti-nociceptive effect of the EOCz, which may contribute to minimising pain and inflammation (Oliveira et al., 2001). The lack of significant alterations on angiogenesis and wound closure observed in the EOCz-treated groups compared to the control groups at day 3 of post-wounding (Figs. 1E and 2B, respectively) was expected, as 3 days is too short a time to observe any effect on the formation of new blood vessels and wound contraction (Velnar et al., 2009, Grieb et al., 2011).

EOCz showed an accentuated capacity for remodelling the healed area, as the analysis of the Masson's Trichrome (MT)-stained sections from the 20% EOCz-treated wound tissue showed an increase in the extracellular matrix deposition, evidenced by the high number of fibroblasts and collagen (Fig. 4C) and also demonstrated accelerated wound closure when compared with the control group at day 15 post-wounding (Fig. 3D). As illustrated in Fig. 5, the dorsum of EOCz-treated mice approached normal architecture tissue at 15 days post-wounding. A similar magnitude in the number of fibroblasts (Fig. 5B and F) and collagen fibres (Fig. 5 H–I) and in the wound closure (Fig. 3D and E) was observed between EOCz- and AT-treated groups, suggesting that AT is fundamental for EOCz wound healing activity, primarily in the underlying molecular mechanism of extracellular matrix remodelling promoted by EOCz treatment. In accordance with our data, previous studies have shown that herbal extracts might enhance the wound repair process by stimulation of fibroblast motility from the wound edges to the wound sites, cellular proliferation and the consequent production of collagen (Fung and Ng, 2005). Atiba et al. (2011) showed that *Aloe vera* accelerated wound contraction in diabetic rats by increasing the migration of fibroblasts and endothelial cells stimulated by transforming growth factor (TGF-β1) and vascular endothelial grown factor (VEGF).

It is noteworthy that the efficacy of wound closure induced by 20% EOCz and 20% anethole were similar to that induced by fibrinolysin (Fibrase®, 5 mg). The analysis of MT-stained sections (Fig. 5) demonstrated that treatments with cataplasms containing 20% EOCz and 20% anethole induced the formation of new fibroblast cells (Fig. 5B and C) and the deposition of collagen fibres (Fig. 5H and I) at an intensity equal to that of the fibrinolysin treatment (Fig. 5G).

Previous reports suggest that the antioxidant and antibacterial properties of EOCz and anethole (Oliveira et al., 2001; Freire et al., 2005; Fontenelle et al., 2008; Coutinho et al., 2010; Shahat et al., 2011) may greatly contribute to optimum cutaneous wound care, as infection caused by microorganisms and oxidative stress can both contribute to the worsening wound healing process. According to reported studies, the healing activity of topical treatment with extracts from *Calendula oficinalis* (Parente et al., 2012) and *Terminalia chebula* (Suguna et al., 2002) was attributed to their anti-inflammatory and antibacterial properties. Similarly, Park et al. (2010) have attributed anti-inflammatory and antioxidant properties to linoleic acid based on the observation of accelerated cutaneous wound healing in mice fed dietary conjugated linoleic acid supplementation.

In summary, our data provide evidence that EOCz and AT act in the inflammatory and remodelling phases of the wound healing process. In addition, these results suggest that AT is directly involved in the effect of EOCz on promoting extracellular matrix remodelling and accelerating the wound closure of injured tissue. Although further investigation will be required to discover the precise mechanism by which EOCz and AT act in the cutaneous wound healing repair, our results are a key to demonstrating the scientific foundation of the traditional and folkloric medical uses of the *Croton zehntneri* plant in terms of its capacity to accelerate and improve the healing process. Additionally, our data show that EOCz is a potential herbal candidate for a safe and effective drug to treat non-healing wounds and other wound complications found in various pathologies, such as diabetes and venous ulcers.

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